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APPLICATION NUMBER: ***60/544,163***
FILING DATE: ***February 13, 2004***
PCT/CA05/00195

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
David Richard	PERRIN TING	Vancouver, CANADA Vancouver, CANADA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Radiolabeled Compositions for PET Imaging, Their Precursors and Methods for their Use

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number:

OR

☑ Firm or Individual Name	The University of British Columbia - Industry Liaison Office				
Address	#103 -6190 Agronomy Road				
Address					
City	Vancouver	State	BC	Zip	V6T 1Z3
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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 10
☐ CD(s), Number _____
☐ Drawing(s) *Number of Sheets* _____
☐ Other (specify) _____
☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees.
☐ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____
☑ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME ANNARI FAURIE

TELEPHONE 604-822-8594

Date 12 February 2004

REGISTRATION NO. _____ *(if appropriate)*

Docket Number: UBC 04-077

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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TO:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Enclosures:

1. Provisional application for patent cover sheet
2. Specifications, 10 pages
3. Credit card payment form PTO-2038 for $80.00 filing fee

THE UNIVERSITY OF BRITISH COLUMBIA

February 12, 2004

Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Re: Provisional Patent Application for "Radiolabeled Compositions for PET Imaging, their Precursors and Methods for their Use"
UBC file no: 04-077

Enclosed please find the necessary documents for filing a Provisional Patent Application for the above-identified technology on behalf of The University of British Columbia. Also enclosed is Credit Card payment form PTO-2038 to cover the cost of the $80.00 application fee.

Thank you,

Sincerely,

Annari Faurie
Patent Manager

Encl.

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Radiolabeled Compositions for PET Imaging, Their Precursors and Methods for their Use.

Introduction

Positron Emission Tomography (PET) is a technology that relies upon the injection of short-lived radionucleotide labeled tracers to examine metabolic processes in the body. Once injected these tracers (which have a relatively short half-life) allow the functioning of the metabolic processes under investigation to be monitored with a PET camera. Changes, potentially caused by the onset of disease can be detected by this technique before the onset of physical changes that can be visualized by scanning techniques such as MRI. The major clinical applications for PET include oncology, neurology, and cardiology.

The invention described herein comprises technologies that relate to the synthesis of a synthon that can:

a) be readily attached to any molecule of choice provided the appropriate linker chemistries, and
b) can be then used to introduce the radioisotope ^{18}F for use in PET studies.

Throughout this application *fluorine and *fluoride represent a composition of fluorine that is isotopically enriched in the ^{18}F isotope. Within equations and figures the symbol *F is used to represent a fluorine (or fluoride *F$^-$) atom in a molecule that is enriched with, or synthesized to newly possess the ^{18}F isotope.

Summary of Invention

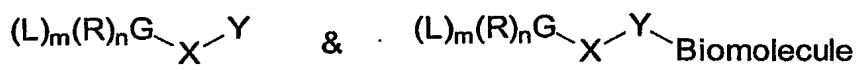
The invention described herein describes radiolabeled compositions that contain either boron-fluorine-18 or silicon-fluorine-18 covalent bonds where the composition is used for PET imaging. Also contemplated are stable precursors to the synthesis of such radiolabeled compositions and methods for producing both the radiolabeled and precursor compositions.

Brief Description of Invention

It is an object of this invention to provide compositions of matter that can readily be chemically transformed such that they incorporate fluorine atoms that are isotopically labeled with ^{18}F. It is a further object of this invention to generate these compositions with an eye to first covalently conjugating them to any so called biomolecule of physiological interest, to form a so-called bioconjugate. Following the optional purification of these bioconjugates and verification of activity in-vitro, these bioconjugates can then be fluorinated in a second step, where the composition of such various compositions of matter provides for quick, quantitative, and facile fluorination.

It is a further object of this invention to provide ^{18}F-radiolabeled compositions for use as imaging agents in positron emission tomography.

In accordance with one aspect of the invention there is provided a composition of one of the following general structures:



Where,

G is either a boron (B) [m = 0 or 1, or 2, or 3; n = 0 or 1, or 2; m + n = 2 or 3] or silicon (Si) [m = 1 or 2 or 3; n = 0 or 1 or 2; m + n = 3] atom.

L are independently suitable leaving groups that can be displaced by fluorine upon treatment with a fluorinating agent, where L is attached to G.

X is an optionally substituted or unsubstituted; linear, branched, or cyclic; saturated or unsaturated, hydrocarbon, heteroatom, or non-carbon containing group that links G to Y.

R is an optionally substituted or unsubstituted; linear, branched, or cyclic carbon skeleton; that may be saturated or unsaturated, optionally interrupted by other non carbon atoms that may be protonated as normal valence bonding would permit, or linked to other atoms as normal valence bonding would permit.

Y is an atom or group of atoms that can form a bond to a so-called biomolecule under appropriately mild conditions.

Biomolecule = a suitable biomolecule or synthetic equivalent of physiological interest as defined below.

Boronation = a general process that refers to the attachment of a boron-containing molecule onto a biomolecule for the eventual and ultimate labeling of the biomolecule with fluoride. Preferred embodiments of this are detailed below.

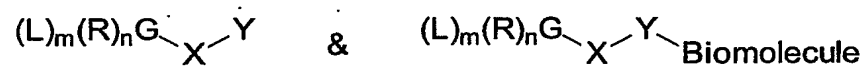
In another aspect of the invention there is contemplated the method of treating these molecules with a fluorinating agent that is enriched in the ^{18}F isotope to form isotopically labeled compositions.

In yet another aspect of this invention there is contemplated the radiolabeled compositions resulting from the fluorination of the precursor molecules.

The use of the isotopically labeled compounds in positron emission tomography (PET) is a further aspect of the invention described herein.

Detailed Description of Invention:

The invention contemplates the precursor molecules of the following general structures.



Where

G is either a boron (B) [m = 0 or 1, or 2, or 3; n = 0 or 1, or 2; m + n = 2 or 3] or silicon (Si) [m = 1 or 2 or 3; n = 0 or 1 or 2; m + n = 3] atom. G is most preferably boron (B).

L are independently suitable leaving groups that can be displaced by fluorine upon treatment with a fluorinating agent. Suitable leaving groups include, but are not limited to, any single, saturated or unsaturated, branched, or linear combination of carbons, hydrocarbons, alkoxides (-OR), hydroxides (-OH) or equivalently alcohols (HOR) or water (H_2O), nitrogen (-NH_2, -NHR, -NR_2, -NHR^+, -NR_2^+, -NH_3^+, -NH_2R^+, -NR_3^+) phosphorus (-PH_2, -PHR, -PR_2, -PHR^+, -PR_2^+, -PH_3^+, -PH_2R^+, -PR_3^+), sulfur (-SH, -SR), sulfone (-SOR), or sulfoxide (-SO_2R) liganded atoms (where R is any chemical group). L may also be either Cl or Br. In the boron case where m =0, m represents a covalently unoccupied pole in the trigonal planar representation of boron which can be occupied by an ^{18}F fluorine atom. In preferred embodiments L = an alkylether group that leaves as either an alkoxides or as an alcohol. In preferred embodiments multiple L may be linked together to form a bi or tridentate ligand to boron. A preferred bidentate leaving group would be $^-$O-Z-O$^-$. Where Z is a saturated or unsaturated, optionally substituted 1 to 4 carbon chain. In the current embodiments Z is -CMe_2-CMe_2-.

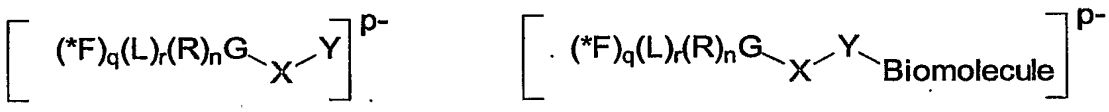
X may be absent or is an optionally substituted or unsubstituted; linear, branched, or cyclic; saturated or unsaturated group that links G to Y. X may incorporate groups of varying composition that include any composition of alkyl chains, aryl rings, amides, esters, ethers, thioethers, sulfoxides, sulfones, amines, heterocycles of with varying compositions of C, N, H, S, O, Cl, Br, I, F, into an optionally substituted, linear or branched, saturated or unsaturated alkyl chain. In preferred embodiments X contains an alkyl, alkenyl, alkynyl, or aromatic group that links to G. The carbon chain of X may be optionally interrupted by one or more O, N, S, or Si atoms as permitted by normal valence bonding rules.

Y is a group that can form a bond to a biomolecule under appropriately mild conditions. Typically Y will contain an electrophilic activating atom, likely to be a carbonyl or a phosphate group, and will react with a nucleophile on the biomolecule such as a nitrogen or sulfur atom. In preferred embodiments Y can be, but is not limited to, an aromatic aldehyde, N-hydroxysuccinimidyl ester group, bromoacetyl or maleimide. Y may be a suitable nucleophile activated in cases where the biomolecule contains the mentioned electrophiles through which conjugation to Y is achieved. In other embodiments, Y may include, but is not limited, to haloacetyls, haloketones, sulfonylhalides, amines of primary, secondary, tertiary nature, alkyl and aryl nitriles, alkyl and aryl azides, alkyl and aryl diazonium salts oximes, hydroxylamines, maleimides, aminoxyls, hydrazines, hydrazides, phosphates, phosphoramidites, phosphines and related trivalent phosphorous compounds, thiophosphates, phosphomorpholidates, phosphoimidazolides, and other activated phosphates, sulfonates, sulfonylhalides, hydroxyls, thiols/mercaptans, thioacids, disulfides, alkylhalides of primary, secondary, tertiary nature, arylhalides, aldehydes, ketones, carboxylic acids and related activated carboxylic acid forms (e.g. NHS esters, HOBt esters, acylpyridiniums, azides, and halides), or any other precursor that can be converted to the aforementioned functionalities for linkage to a so called biomolecule.

R are independently aliphatic (alkyl) $(CH_2)_s$ (s = to 0) or aryl (C_6H_5) groups optionally interrupted by oxygen (-O-) groups or aryl (C_6H_5) groups substituted by a zero to 5 number of hydroxyl, alkyl, aryl, thio, thioether, amino, azo, hydrazino, ester, amide, carboxyl, carboxylate, phosphate, sulfoxide and/or sulfonate groups. The saturated or unsaturated chain of R may be optionally and independently substituted by any number of hydroxyl, alkyl, aryl, thio, thioether, amino, azo, hydrazino, ester, amide, carboxyl, carboxylate, phosphate, sulfoxide and/or sulfonate groups. Alternatively, R may include primary (NR'), secondary (NR_2'), or tertiary (NR_3'$^+$) amine, imide, or imid group (not excluding any nitrogen containing heterocycles), which may be substituted by any number of a hydroxyl, alkyl, aryl, thio, thioether, amino, ester, amide, carboxyl carboxylate, phosphate, sulfoxide and/or sulfonate groups.

Biomolecule is a biomolecule, or analog or derivative of a biomolecule, or other molecule that may be delivered into a human or animal in order to track or image the biomolecule's distribution within the human or animal via positron emission tomography. In preferred embodiments the word "biomolecule" pertains to any molecule of medical or scientific significance. Preferred examples of so called biomolecules may include, but are not limited to, peptide hormones, antibodies, aptamers and oligonucleotides, proteins, peptides, oligodeoxyribonucleotides, lipids, hormones, drugs, polysaccharides, liposomes, micelles, microsomes, magnetic particles, metal chelators, oligoribonucleotides, oligonucleotides and related analogs bearing modifications in the backbone, nucleobase, or phosphate linker regions that enhance stability or modulate specificity, peptidomimetics, dendrimers, drug delivery agents, nanotubes, fullerenes, virus particles.

Also contemplated within the scope of this invention are radiolabeled compositions of the following general formula.



Where

G is either a boron (B) [q = 1, 2, or 3; r = 0 or 1, or 2; n = 0 or 1, or 2; q + m + n = 2 or 3] or silicon (Si) [q = 1, 2, or 3; r = 1 or 2; n = 0 or 1, or 2; q + m + n = 3] atom. G is most preferably boron (B).

L are independently suitable leaving groups that can be displaced by fluorine upon treatment with a fluorinating agent. Suitable leaving groups include but are not limited to, any single, saturated or unsaturated, branched, or linear combination of carbons, hydrocarbons, alkoxides (-OR), hydroxides (-OH) or equivalently alcohols (HOR) or water (H_2O), nitrogen ($-NH_2$, -NHR, $-NR_2$, $-NHR^+$, $-NR_2^+$, $-NH_3^+$, $-NH_2R^+$, $-NR_3^+$) phosphorus ($-PH_2$, -PHR, $-PR_2$, $-PHR^+$, $-PR_2^+$, $-PH_3^+$, $-PH_2R^+$, $-PR_3^+$), sulfur (-SH, -SR), sulfone (-SOR), or sulfoxide ($-SO_2R$) liganded atoms (where R is any chemical group). L may also be either Cl or Br. In the boron case where m = 0, m represents a covalently unoccupied pole in the trigonal planar representation of boron which can be occupied by an ^{18}F fluorine atom. In preferred embodiments r is zero and no L are present and boron is recognized as being tetravalent with the conjugate being considered an organotrifluoroborate in the preferred embodiment.

X may be absent or is an optionally substituted or unsubstituted; linear, branched, or cyclic; saturated or unsaturated group that links G to Y. X may incorporate groups of varying composition that include any composition of alkyl chains, aryl rings, amides, esters, ethers, thioethers, sulfoxides, sulfones, amines, heterocycles of with varying compositions of C, N, H, S, O, Cl, Br, I, F, into an optionally substituted, linear or branched, saturated or unsaturated alkyl chain. In preferred embodiments X contains an alkyl, alkenyl, alkynyl, or aromatic group that links to G. The carbon chain of X may be optionally interrupted by one or more O, N, S, or Si atoms as permitted by normal valence bonding rules.

Y is a group that can form a bond to a biomolecule under appropriately mild conditions. Typically Y will contain an electrophilic activating atom, likely to be a carbonyl or a phosphate group, and will react with a nucleophile on the biomolecule such as a nitrogen or sulfur atom. In preferred embodiments Y can be, but is not limited to, an aromatic aldehyde, N-hydroxysuccinimidyl ester group, bromoacetyl or maleimide. Y may be a suitable nucleophile activated in cases where the

biomolecule contains the mentioned electrophiles through which conjugation to Y is achieved. In other embodiments, Y may include, but is not limited, to haloacetyls, haloketones, sulfonylhalides, amines of primary, secondary, tertiary, or armomatic nature, oximes, hydroxylamines, maleimides, aminoxyls, hydrazines, alkyl and aryl diazonium salts, alkyl and aryl nitriles, alkyl and aryl azides hydrazides, phosphates, phosphoramidites, phosphines, H-phosphonates and related trivalent phosphorous compounds, thiophosphates, activated phosphates such as but not limited to phosphomorpholidates and phosphoimidazolides, as well as other activated phosphates, sulfonates, sulfonylhalides, hydroxyls, thiols/mercaptans, thioacids, disulfides, alkylhalides of primary, secondary, tertiary nature, arylhalides, aldehydes, ketones, carboxylic acids and related activated carboxylic acid forms (e.g. NHS, HOBt esters, acylpyridiniums, azides, and halides) or any other precursor that can be converted to the aforementioned functionalities for linkage to a so called biomolecule.

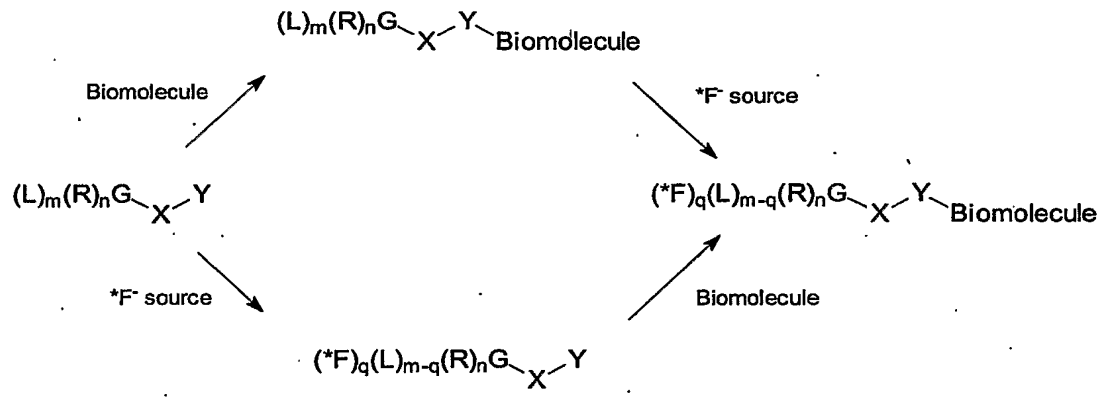
R are independently aliphatic (alkyl) $(CH_2)_s$ (s = 0 to 12) or aryl (C_6H_5) groups optionally interrupted by oxygen (-O-) groups or aryl (C_6H_5) groups substituted by a zero to 5 number of hydroxyl, alkyl, aryl, thio, thioether, amino, azo, hydrazino, ester, amide, carboxyl, carboxylate, phosphate, sulfoxide and/or sulfonate groups. The saturated or unsaturated chain of R may be optionally and independently substituted by any number of hydroxyl, alkyl, aryl, thio, thioether, amino, azo, hydrazino, ester, amide, carboxyl, carboxylate, phosphate, sulfoxide and/or sulfonate groups. Alternatively, R may include primary (NR'), secondary (NR_2'), or tertiary ($NR_3'^+$) amine, imide, or imid group (not excluding any nitrogen containing heterocycles), which may be substituted by any number of a hydroxyl, alkyl, aryl, thio, thioether, amino, ester, amide, carboxyl carboxylate, phosphate, sulfoxide and/or sulfonate groups.

Biomolecule is defined as a biomolecule, or analog or derivative of a biomolecule, or other molecule that may be delivered into a human or animal in order to track or image the biomolecule's distribution within the human or animal via PET (positron emission tomography). In preferred embodiments the word "biomolecule" pertains to any molecule of medical or scientific significance. Preferred examples of so called biomolecules may include, but are not limited to, peptide hormones, antibodies, aptamers and oligonucleotides, proteins, peptides, oligodeoxyribonucleotides, lipids, steroid and nonsteroid hormones, synthetic drugs and natural products, polysaccharides, liposomes, micelles, microsomes, magnetic particles, metal chelators, oligoribonucleotides, oligonucleotides and related analogs bearing modifications in the backbone, nucleobase, or phosphate linker regions that enhance stability or modulate specificity, peptidomimetics, dendrimers, drug delivery agents, nanotubes, fullerenes, virus particles.

Also contemplated within the scope of this invention are methods for transforming the precursor molecules into radiolabeled compounds suitable for use in positron emission tomography studies. The precursor molecules are transformed into the radiolabeled analogues by way of their reaction with a suitable source of ^{18}F isotopically enriched fluorine. Such sources include, but are not limited to, HF, KF, KHF_2, ^{18}F-enriched metal-fluorine salts, ^{18}F-enriched salts of quaternary nitrogenous bases (such as $(Bu)_4NF$), or solutions thereof; and presynthesized derivatives of ^{18}F-labeled boron or silicon (such as $Et_2O.B^*F_3$, Isotopically labeled trifluoroboron etherate). The fluorinating agent may contain any cosolvents including but not limited to THF, DMF, formamide, DMSO, water, methanol, ethanol, DMA, pyridine. Following labeling, excess ^{18}F may be sequestered by addition of other components including but not limited to sliver salts, silicates or silanes, and other activated silicon-derived molecules, boronic esters or boronic acids, such that these additives react to complex free fluoride and where the complexation reaction is then removed by extraction, precipitation, gel-permeation, or other purificative/separative process. Also contemplated for fluorination is to link the precursor bioconjugate to a solid support presenting the

diol functionality (examples include but are not limited to dextran, sephadex, polymerized/cross linked starch, paper, cellulose, or any diol that is modified with a small tight binding ligand (e.g. biotin) that can be captured by a large molecule receptor (e.g. avidin) on solid support) where the linkage between the bioconjugate and the solid support is a boronic ester linkage, or other related bidentate linkage to boron in keeping with stated compositions for L and R. Fluorination would promote release of the labeled biomolecule that would acquire the trifluoroborate component upon release. This is envisioned to increase the specific radioactivity during fluorination of the biomolecule and to enhance purity of the fluorinated/labeled form leaving residual unlabeled species attached to a solid support, in embodiments and applications where this approach is viable.

As shown in the scheme below, the final useful composition of *F-linker-biomolecule may be formed either via the preformation of the linker-biomolecule compound followed by reaction with a *fluorinating source, or by the preformation of the *F-linker compound followed by reaction with the biomolecule. The former method is preferred for two reasons: 1) ability to prepare, purify, and analyze the precursor bioconjugate in bulk to ensure effective coupling and retain bioactivity prior to labeling and 2) ability to minimize reaction steps following the incorporation of radiolabel, a consequence that is desirable with regard to both safety issues in handling the material and the relatively short half-life of ^{18}F.

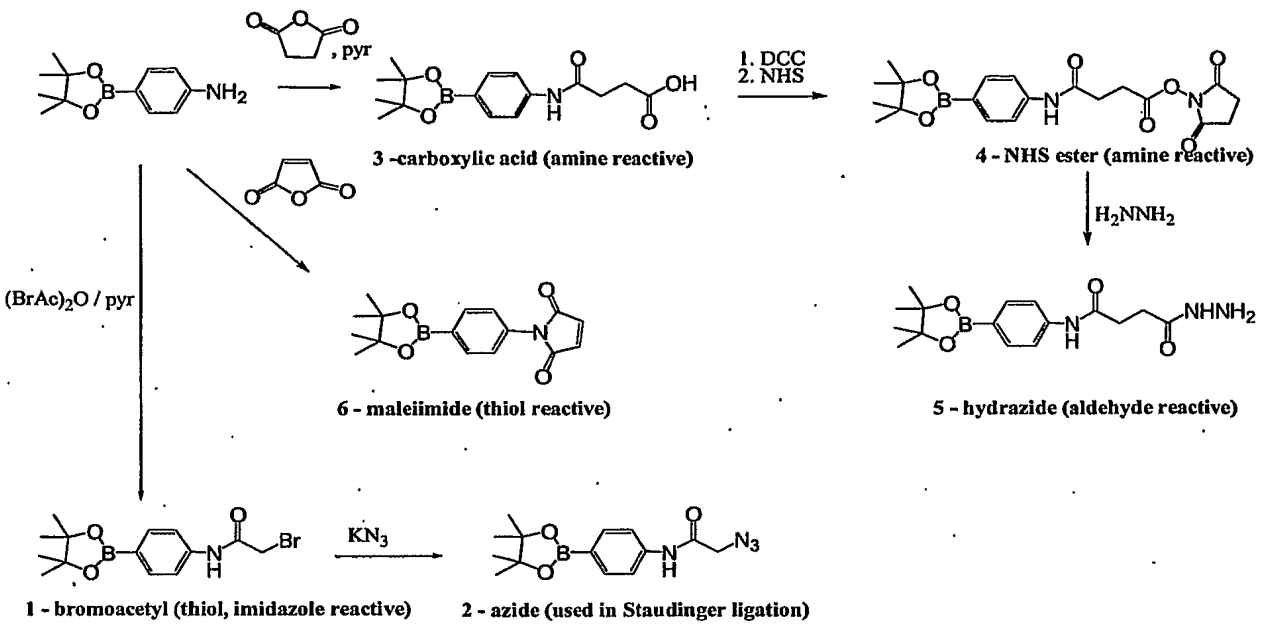


Examples

The following examples are included to illustrate some of the embodiments of the invention described herein. These examples should not be considered to limit the spirit or scope of the invention in any way.

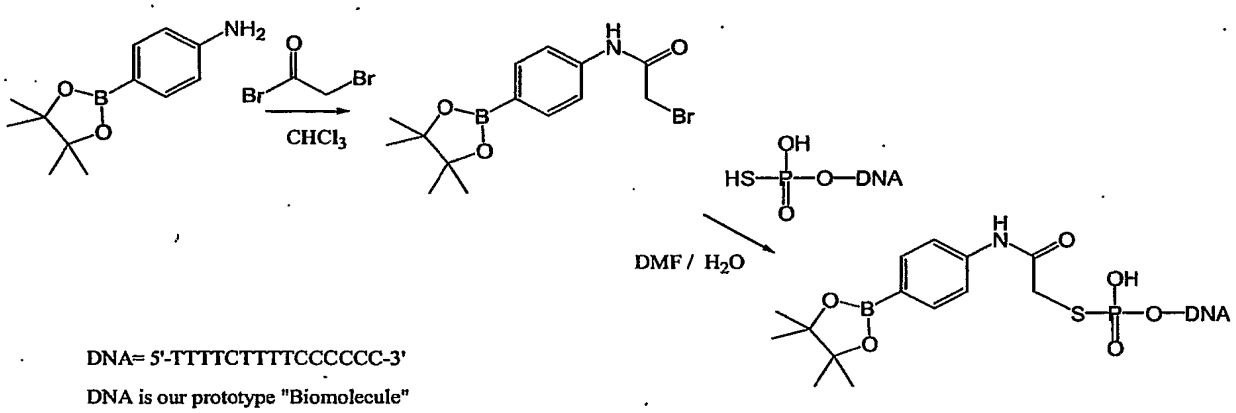
Example 1:

The following scheme provides examples (compounds 1 to 6) of precursor compounds that have the appropriate chemical functionality that will allow them to both be fluorinated at the boron atom (upon treatment with an appropriate fluorinating agent) and also react with various reactive sites on biomolecules.



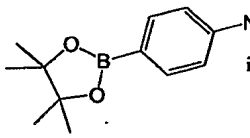
Example 2.

The following scheme illustrates a portion of the synthetic route taken in the synthesis of a prototype precursor molecule containing a boronic ester that can be fluorinated.



DNA= 5'-TTTTCTTTTCCCCCC-3'

DNA is our prototype "Biomolecule"



is our prototype Boron containing molecule

Example Materials and Methods:

The synthetic procedures described herein are given for the purposes of example and illustration only and should not be considered to limit the spirit or scope of the invention

1) The Synthesis and Stability of a ^{19}F-Boron prototype:

4-Ammoniumphenyl Trifluoroborate. A saturated solution of 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl) aniline was made up in of methanol (300 µL, reagent grade). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.45 (d, J= 8 Hz, 2H), δ 6.62 (d, J=8 Hz, 2H), δ 4.84 (s, 2H), δ 1.28 (s, 12H). ^{11}B NMR (400 MHz, MeOH-d_4, BF_2OEt_2 ref) δ 31.13 (s). Upon room temperature addition of an aqueous 48 % HF solution (100 µL, 2.76 mmol), instantaneous formation of a white precipitate was observed. This white precipitate was filtered and washed three times with 300 µL of ethanol. The solid had a pH of 1 when dissolved in 300 µL water. ^{1}H NMR (400 MHz, D_2O) δ 7.55 (d, J= 8 Hz, 2H), δ 7.20 (d, J=8 Hz, 2H). ^{11}B NMR (400 MHz, D_2O, BF_2OEt_2 ref) δ 3.57 (s). ^{19}F NMR (300 MHz, D_2O, TFA ref) δ -53.52 (s) δ -65.57 (s). ESI (negative mode) m/z calcd for $C_6H_6BF_3N^-$ 160.0, found 159.8.

2) The Construction of a Thiophilic Boronating Reagent:

4-(2-Bromoacetamido (4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)Benzene. 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (100 mg, 0.46 mmol) was dissolved in CH_2Cl_2 (1 mL, dried over CaH_2). Bromoacetyl bromide (44 µl, 0.51 mmol) was added to this solution while stirring at room temperature. This solution was stirred at room temperature for an additional 30 min before being diluted with 9 mL more $CHCl_3$. The resulting mixture was washed three times with 10 mL of water. The final wash had a pH of 5.5. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum. The resulting solid was a beige powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), δ 7.77 (d, J=8 Hz, 2H), δ 7.53 (d, J=8 Hz, 2H), δ 3.98 (s, 2H), δ 1.29 (s, 12H). ^{11}B NMR (400 MHz, D_2O, BF_2OEt_2 ref) δ 31.36 (s).

3) The Synthesis of a Boron-based DNA Prototype ^{18}F Synthon:

An oligodeoxyribonucleotide (ODN) of sequence 5'-TTTTCTTTTCCCCCC-3' bearing a 5' thiophosphate was synthesized using standard automated solid-phase methods on applied Biosystems DNA synthesizers by the Nucleic Acids and Protein Synthesis (NAPS) unit at UBC.

A 1 mM solution of the ODN (20 µL H_2O solution, 20 nmol), Tris(2-carboxyethyl)-phosphine HCl (3.6 µl H_2O solution, 100 nmol, pH adjusted to 7.0 with Triethylamine, PIERCE chemicals) was added. This solution was allowed to sit at room temperature for 5 min before 4-(2-Bromoacetamido (4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)Benzene (3.6 µL, 120 nmol) was added. This mixture was allowed to sit at room temperature for 16 hours.

The reaction was desalted over a G-25 spin column. Purification of the boronic-ester-containing ODN conjugate was achieved by drying down the desalted ODN and resuspending it in 30 µl of a 10 mM EDTA (pH 8), 95% formamide, 4% H_2O, 0.5% bromophenol blue 0.5% xylene cyanol, then loading the solution onto a 7 M urea 20 % 29:1 bis: acrylamide polyacrylamide (D-PAGE) gel. DNA was imaged through UV shadowing and eluted from gels by using the crush and soak method with 1 % $LiClO_4$/ 0.7 mM NEt_3. The eluant was dried, resuspended in water and precipitated with 1% $LiClO_4$ in acetone. They were washed twice with ethanol before being desalted over a G-25 spin column.

^{32}P-labeling of this ODN was done with Terminal Deoxynucleotide Transferase (New England Biolabs) and α ^{32}P ddATP. PAGE analysis of the boronation reaction revealed that more than 80% of the thiophosphate ODN was converted to the boronic-ester-conjugated ODN. The relative mobility of the boronic-ester-conjugated ODN was retarded against the unlabeled thiophosphate ODN control. The synthesis of the thiophosphate ODN by NAPS was not clean as an extra band, which could not be labeled, was observed in both the control and the boronic-ester-conjugated ODN lanes in the PAGE analysis.

4) Initial Kinetic protocol Investigations for optimal Boron-based ^{18}F-DNA labeling

Prior to ^{18}F-labeling on the final DNA compound, dilute labeling conditions were developed for 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl) aniline with cold ^{19}F and the expectation that these labeling conditions can be extrapolated onto ^{18}F studies. The general conditions established are as follows: 200 mM Acetic acid at pH 3.5, with 2 mM boron compound, and 20 mM KHF_2. In these conditions, between 2 and 3 fluorine atoms were picked up by the small boron molecule (ϕBF_3). ^{19}F NMR (300 MHz, D_2O, TFA ref) δ -53.89 (s, KHF_2, 77 % of fluorine integral), δ -62.50 (s, ϕBF_3, 33% of fluorine integral). Spectroscopic studies showed a protecting group hydrolysis rate of 0.66 $\pm$ 0.04 min^{-1} in these conditions. Kinetics of fluorination in 100 mM Acetic acid at pH 3.5 showed that the rate of fluorination was 2.8 $\pm$ 0.3 min^{-1} in these conditions. The persistence of a ^{19}F NMR peak near δ -66 despite a 20 mM boric acid chase, or in fluorination in the presence of 20 mM boric acid indicates that the B-F ϕBF_3 bond is stable. Fluorination after a 20 mM boric acid chase (chase at 1h, NMR taken at 2h): ^{19}F NMR (300 MHz, D_2O, TFA ref) δ -50.23 (fluorine-boric acid species #1), δ -53.89 (KHF_2), δ -62.3 (fluorine-boric acid species #2), δ -66.30 (s, ϕBF_3). Fluorination in the presence of boric acid: ^{19}F NMR (300 MHz, D_2O, TFA ref) δ -50.02 (fluorine-boric acid species #1), δ -52.47 (KHF_2), δ -62.0 (fluorine-boric acid species #2), δ -65.65 (s, ϕBF_3).

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